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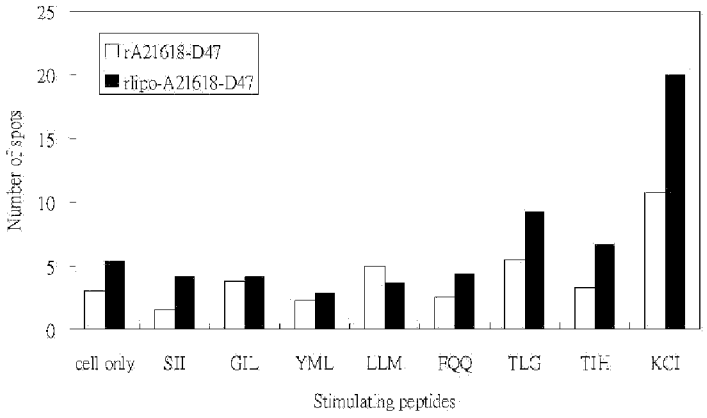
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(54) Title: LIPIDATED POLYEPITOPE VACCINES

FIG. 3



(57) Abstract: This invention relates to, *inter alia*, an isolated lipidated polypeptide including a lipid moiety at the N-terminus and a plurality of epitopes, and methods of making and using the polypeptide.

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LIPIDATED POLYEPITOPE VACCINES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims priority to US Provisional Application No. 61/413,619, filed November 15, 2010, the content of which is incorporated herein by reference in its entirety.

BACKGROUND

The use of epitopes identified from pathogens as target antigens is an important strategy for developing therapeutic vaccines. Immunization with polyepitopes would provide advantages over single epitope-based vaccines. Thus far, development of polyepitope vaccines has mainly been focused on DNA vaccines. However, there are various safety concerns regarding DNA vaccines. There is a need to develop polyepitope vaccines that can overcome the obstacle of DNA-based vaccines.

SUMMARY

This invention is based on the discovery that lipidated polypeptides containing multiple epitopes, when administered to a subject, can induce an immune response in the subject.

In one aspect, described herein is an isolated lipidated polypeptide including a lipid moiety at the N-terminus and a plurality of epitopes. Each epitope of the plurality can be different. In some embodiments, all epitopes of the plurality are cytotoxic T lymphocyte (CTL) epitopes.

All epitopes of the plurality can be from a single pathogen, e.g., human papillomavirus (HPV). In some cases, the plurality of epitopes include one or more epitopes on E6 or E7. In some cases, the plurality of epitopes include different HLA-A2-restricted epitopes of HPV type-16 and type-18, e.g., one or more or all of the peptides listed in Table 1. In some embodiments, the epitopes of the plurality are contiguous or noncontiguous. In some embodiments, the polypeptide is rlipo-A21618-D47 or rlipo-A21618-SDSK.

In another aspect, a composition comprising the lipidated polypeptide described herein is contemplated.

In yet another aspect, also described herein is a method of producing the lipidated

polypeptide. The method includes providing an *E. coli* host cell that has an expression vector including a nucleic acid encoding a polypeptide, the polypeptide having a bacterial lipoprotein signal peptide and the plurality of epitopes; and culturing the *E. coli* host cell to allow expression of the polypeptide in lipidated form, thereby producing the lipidated polypeptide. In some embodiments, the nucleic acid is codon-optimized for expression in E. coli. In some cases, the lipoprotein signal peptide comprises the D1 domain of Ag473.

In yet another aspect, described herein is a method of eliciting an immune response in a subject, the method includes administering to the subject a composition containing the lipidated polypeptide. The immune response can be a CTL-mediated immune response.

The invention also contemplates a method for treating a human papillomavirus (HPV)-associated disease. The method includes administering to a subject in need thereof an effective amount of a composition containing the lipidated polypeptide described herein.

Also included in this invention is a use of the above-described polypeptide or composition for eliciting an immune response or for treating an HPV-associated disease in a subject.

An "antigen" refers to a molecule containing one or more epitopes that will stimulate a host's immune system to make a humoral and/or cellular antigen-specific response. The term "antigen" is used interchangeably with "immunogen." As a result of coming in contact with appropriate cells, an "antigen" induces a state of sensitivity or immune responsiveness and reacts in a demonstrable way with antibodies or immune cells of the sensitized subject in vivo or in vitro. An "antigen" can be specifically recognized and bound by antibodies in an organism. An antigen in association with a major histocompatibility complex (MHC) can also be recognized and bound by receptors on the surface of T lymphocytes (T-cells), leading to the activation of the T-cells.

The term "epitope" as used herein refers to the site on an antigen recognized by antibodies or immune cells, e.g., B-cells and T-cells. The term "epitope" is used herein interchangeably with "antigenic determinant" or "antigenic determinant site." A cytotoxic T-cell (CTL) epitope refers to an epitope capable of activating a CTL (also known as Tc or killer T cell), which subsequently stimulates CTL responses, i.e., inducing death of abnormal cells (e.g.,

virus-infected or tumor cells). A CTL epitope, typically including 8-11 amino acid residues, forms a complex with a particular MHC class I molecule (including a heavy chain and a β2 microglobulin) presented on the surface of an antigen-presenting cell. This complex, upon binding to a T cell receptor of a CD_8 T cell (a CTL), activates the T cell, thus triggering CTL responses.

The term "immune response" or "immunogenic response" refers to any reaction of the immune system in response to an antigen in a subject. Examples of an immune response in a vertebrate include, but are not limited to, antibody production, induction of cell-mediated immunity, complement activation, and development of immune tolerance. The immune response to a subsequent stimulus by the same antigen, also named the secondary immune response, can be more rapid than in the case of the primary immune response.

A “subject” refers to a human and a non-human animal. Examples of a non-human animal include all vertebrates, e.g., mammals, such as non-human primates (particularly higher primates), dog, rodent (e.g., mouse or rat), guinea pig, cat, and non-mammals, such as birds, amphibians, etc. In a preferred embodiment, the subject is a human. In another embodiment, the subject is an experimental animal or animal suitable as a disease model.

The term “treating” as used herein refers to the application or administration of a composition including one or more active agents to a subject, who has a disease, a symptom of the disease, or a predisposition toward the disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptoms of the disease, or the predisposition toward the disease. “An effective amount” as used herein refers to the amount of each active agent required to confer therapeutic effect on the subject, either alone or in combination with one or more other active agents. Effective amounts vary, as recognized by those skilled in the art, depending on route of administration, excipient usage, and co-usage with other active agents.

The term “isolated” as used herein with reference to a polypeptide refers to a polypeptide substantially free from naturally associated molecules; namely, it is at least 60% (i.e., about 65, 70, 75, 80, 85, 90, 95, or 99 percent) pure by dry weight. Purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, and

HPLC.

The term "lipoprotein signal peptide" or "lipid signal peptide" refers to a peptide found on naturally-occurring lipoprotein or a variant thereof that facilitates lipidation of a polypeptide carrying the signal peptide at its N-terminus.

The details of one or more embodiments of the invention are set forth in the accompanying drawing and the description below. Other features, objects, and advantages of the invention will be apparent from the description and drawing, and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a table showing the amino acid sequences of rA21618-D47 (SEQ ID NO:1) and rlipo-A21618-D47 (SEQ ID NO:2). The underlined portion is the sequence of rA21618-D47. rlipo-A21618-D47 includes the amino acid sequence of rA21618-D47 plus a lipid signal peptide at the N-terminus.

FIG. 2 is a table showing the amino acid sequences of rA21618-SDSK (SEQ ID NO:3) and rlipo-A21618-SDSK (SEQ ID NO:4). The underlined portion is the sequence of rA21618-SDSK. rlipo-A21618-SDSK includes the amino acid sequence of rA21618-SDSK plus a lipid signal peptide at the N-terminus.

FIG. 3 is a bar graph showing induction of epitope-specific CTL response in HLA-A2-transgenic mice by rA21618-D47 and rlipo-A21618-D47.

FIG. 4 is a bar graphs showing induction of epitope-specific CTL response in HLA-A2-transgenic mice by rA21618-SDSK and rlipo-A21618-SDSK.

DETAILED DESCRIPTION

Described herein is a lipidated polypeptide including a lipid moiety at the N-terminus and a plurality of epitopes, and methods of making and using them.

The lipidated polypeptide of the present invention includes a plurality of epitopes, e.g., at least two. The epitopes in the lipidated polypeptide can be contiguous or separated by linker amino acid sequences. Each linker in a lipidated polypeptide can be the same or different. A linker can include up to 8 amino acids (e.g., 1, 3, 6 or 8). Those of ordinary skill in the art would

appreciate that there are many possible configurations of epitopes and linkers.

The epitopes of the lipidated polypeptide can be any epitopes known in the art. Epitopes identified using methods known in the art can also be used to generate the lipidated polypeptide. The plurality of epitopes in a particular lipidated polypeptide can be all from the same pathogen. In some embodiments, the plurality of epitopes are CTL epitopes. Examplary epitopes useful for the present invention include those described in U.S. Application Pub. No. 2009/0117123, U.S. Pat. Application No. 12/787,539, Duraiswamy et al. (2003, J. Virology. 77:7401-10) and Lutzky et al. (2010 J. Virology. 84:407-417).

The lipidated polypeptide of the invention can be obtained as a synthetic polypeptide or a recombinant polypeptide. A lipoprotein signal peptide or lipid signal peptide can be linked to a target polypeptide, e.g., one that contains a plurality of epitopes, by conventional recombinant technology to form a fusion protein, which is in lipidated form when expressed in *E. coli*. Examples of lipoprotein signal peptides include those described in U.S. Pat. Pub. No. 2009/0221499.

For example, to produce a lipidated polypeptide, a DNA fragment encoding the lipoprotein signal peptide and a DNA fragment encoding the target polypeptide are inserted into an expression vector, preferably carrying a strong promoter (e.g., T7, T5, T3, or SP6), to construct an expression plasmid. The strong promoter can be inducible, e.g., by isopropyl β-D-thiogalactoside (IPTG). The expression plasmid is then introduced into an *E. coli* host strain and positive transformants are cultured under suitable conditions for protein expression. It is preferred that the *E. coli* host strain be resistant to the toxic effects induced by over-expression of exogenous proteins. Such *E. coli* strains can be identified or generated by the methods described in US Patent No. 6,361,966. Examples of these *E. coli* strains include, but are not limited to, C43(DE3) (ECCC B96070445), C41(DE3) (ECCC B96070444), C0214(DE3), DK8(DE3)S (NCIMB 40885), and C2014(DE3) (NCIMB 40884).

Preferably, the fusion protein thus expressed is isolated from the *E. coli* host cells and its lipidation status is confirmed via methods known in the art, e.g., immunoblotting with an anti-lipoprotein antibody or mass spectrometry.

Useful lipoprotein signal peptides include the signal peptide of *E. coli* acriflavine-

resistance protein E precursor and the signal peptide of *Neisseria meningitides* Ag473 protein. Lipoprotein signal peptides can be identified by, for example, identifying the signal peptide on natural lipoproteins (e.g., those listed in the DOLOP database at mrc-lmb.cam.ac.uk/genomes/dolop/). Variants of naturally-occurring lipoprotein signal peptides can also be used.

Also described herein is a composition including the lipidated polypeptide of the present invention. The composition can be prepared according to any methods known in the art. For example, the composition can contain an effective amount of the lipidated polypeptide of the invention, and a pharmaceutically acceptable carrier such as a phosphate buffered saline, a bicarbonate solution, or an adjuvant. The carrier must be "acceptable" in the sense that it is compatible with the active ingredient of the composition, and preferably, capable of stabilizing the active ingredient and is not deleterious to the subject to be treated. The carrier is selected on the basis of the mode and route of administration, and standard pharmaceutical practice. Suitable pharmaceutical carriers and diluents, as well as pharmaceutical necessities for their use, are described in Remington's Pharmaceutical Sciences. An adjuvant, e.g., a cholera toxin, *Escherichia coli* heat-labile enterotoxin (LT), liposome, immune-stimulating complex (ISCOM), or immunostimulatory sequences oligodeoxynucleotides (ISS-ODN), can also be included in a composition of the invention, if necessary. The composition can also include a polymer that facilitates *in vivo* delivery. See Audran R. et al. Vaccine 21:1250-5, 2003; and Denis-Mize et al. Cell Immunol., 225:12-20, 2003.

The above-described lipidated polypeptide can be used in an immunogenic composition, e.g., a vaccine, for generating antibodies and immune response against a pathogen in a subject susceptible to the pathogen. Exemplary pathogens include, but are not limited to human papillomavirus (HPV), hepatitis C virus (HCV), Epstein-Barr virus (EBV), herpes simplex virus 1 (HSV-1), herpes simplex virus 2 (HSV-2), cytomegalovirus (CMV), respiratory syncytial virus (RSV), parainfluenza virus type 3 (PIV3), influenza viruses, dengue virus, west Nile virus, Norovirus, and SARS coronavirus. Methods for preparing vaccines are well known in the art, as exemplified by U.S. Patents. 4,601,903; 4,599,231; 4,599,230; and 4,596,792. Vaccines can be prepared as injectables, as liquid solutions or as emulsions. The lipidated polypeptide of this

invention may be mixed with physiologically acceptable excipients. Excipients can include, water, saline, dextrose, glycerol, ethanol, and combinations thereof. The vaccine can further contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, or an adjuvant to enhance the effectiveness of the vaccines. Methods of achieving adjuvant effect for the vaccine include use of agents, such as aluminum hydroxide or phosphate (alum), commonly used as 0.05 to 0.1 percent solutions in phosphate buffered saline. Vaccines can be administered parenterally, subcutaneously or intramuscularly. Alternatively, other modes of administration including suppositories and oral formulations may be desirable. For suppositories, binders and carriers can include, for example, polyalkalene glycols or triglycerides. Oral formulations can include normally employed incipients such as pharmaceutical grade saccharine, cellulose, magnesium carbonate and the like. These compositions can take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10-95% of the immunopeptide described herein.

The vaccine is administered in a manner compatible with the dosage formulation, and in an amount that is therapeutically effective, protective and immunogenic. The quantity to be administered depends on the subject to be treated, including, for example, the capacity of the individual's immune system to synthesize antibodies, and if needed, to produce a cell-mediated immune response. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner. However, suitable dosage ranges are readily determinable by one skilled in the art and can be of the order of micrograms of the polypeptide of this invention. Suitable regimes for initial administration and booster doses are also variable, but may include an initial administration followed by subsequent administrations. The dosage of the vaccine may also depend on the route of administration and varies according to the size of the host.

For example, a subject susceptible to HPV infection can be identified and administered the composition described above. The dose of the composition depends, for example, on the particular lipidated polypeptide, whether an adjuvant is co-administered with the lipidated polypeptide, the type of adjuvant co-administered, and the mode and frequency of administration, as can be determined by one skilled in the art. Administration is repeated, if necessary, as can be determined by one skilled in the art. For example, a priming dose can be

followed by three booster doses at weekly intervals. A booster shot can be given at 4 to 8 weeks after the first immunization, and a second booster can be given at 8 to 12 weeks, using the same formulation. Serum sample or T-cells can be taken from the subject to determine the immune response elicited by the vaccine against the HPV E6 or E7 protein. Methods of assaying cytotoxic T cells against a protein or infection are well known in the art. Additional boosters can be given as needed. By varying the amount of the lipidated polypeptide, the dose of the composition, and frequency of administration, the immunization protocol can be optimized for eliciting a maximal immune response. Efficacy of the composition may also be tested. In an efficacy testing, a non-human subject can be administered via an oral or parenteral route with a composition of the invention. After the initial administration or after optional booster administration, both the test subject and the control subject (receiving mock administration) are challenged with an HPV. End points other than lethality can be used. Efficacy is determined if subjects receiving the composition are free from HPV infection or develop symptoms associated with HPV infection at a rate lower than control subjects. The difference should be statistically significant.

The specific example below is to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present invention to its fullest extent. All publications cited herein are incorporated by reference in their entirety.

Example

Whether lipidated polypeptides containing polyepitopes could induce epitope-specific immune responses was investigated. Several HLA-A2-restricted CTL epitopes of HPV type-16 and type-18 were used to construct polyepitope vaccines. A lipid signal peptide was added to the N-terminus of the polypeptides to form the lipidated polyepitope vaccines, rlipo-A21618-SDSk and rlipo-A21618-D47. These recombinant polyepitopes vaccines contain epitopes of HPV type-16 and type-18, which are major pathogens that cause cervical cancer. HLA-A2-transgenic mice were immunized subcutaneously with these lipidated polyepitope vaccines. Data show that both rlipo-A21618-SDSk and rlipo-A21618-D47 can induce epitope-specific CTL response in

HLA-A2-transgenic mice.

The selection of epitopes was based on the previous identified HPV16 and HPV18 CTL epitopes for HLA-A2. See Table 1. The extended N- and C-terminal six amino acids flanking each epitope were also selected. All epitopes and their flanking region were concatenated in different order to form A21618-D47 and A21618-SDSK. See, Figs. 1 and 2. In the case of A21618-SDSK, a helper T cell epitope (PADRE), one mouse MHC class I epitope, and an HLA-A2 epitope from influenza A matrix protein were also included. In order to improve the expression of polyepitope proteins in *E. coli*, some amino acids were changed according to the hydropathy analysis using TMHMM, PSORTb, and SOSUI software. A lipobox-containing signal peptide was added to the N-terminus of the polyepitopes proteins to form lipidated polyepitopes. The DNA sequences of both polyepitopes were synthesized *in vitro* by PRISMA Biotech Corporation.

TABLE 1. Names and sequences of epitopes

Name	Source	Sequence
SII	Mouse control epitope	SIINFEKL (SEQ ID NO:5)
GIL	HLA-A2 control epitope	GILGFVFTL (SEQ ID NO:6)
YML	HPV16	YMLDLQPETT (SEQ ID NO:7)
LLM	HPV16	LLMGTLGIV (SEQ ID NO:8)
FQQ	HPV18	FQQLFLNTL (SQ ID NO:9)
TLG	HPV16	TLGIVCPI (SEQ ID NO:10)
TIH	HPV16	TIHDIILECV (SEQ ID NO:11)
KCI	HPV18	KCIDFYSRI (SEQ ID NO:12)

The A21618-D47 nucleotide sequence was amplified using the forward primer, 5'-GGAATTCCATATGACCCCGACCCTGCA-3' (SEQ ID NO:13), which contains an NdeI restriction site, and the reverse primer, 5'-CCGCTCGAGCGGTTTCTGGCTGCAAATC-3' (SEQ ID NO:14), which is complementary to the coding sequence and contains an XhoI restriction site. The 5' primer for amplification of the A21618-SDSK nucleotide sequence was 5'-CGGGATCCATGAGCGCGGCGAAATTTG-3' (SEQ ID NO:15) and the 3' primer was 5'-

CCGCTCGAGCCACGGACACACAAAG-3' (SEQ ID NO:16). The PCR products were cloned into the expression vector pET-22b (+). To express proteins, the plasmid was transformed into the *E. coli* BL21 (DE3) strains and the bacteria were incubated at 37°C overnight. The expression of rA21618-D47 and rA21618-SDSK were induced with 1mM IPTG for 3-4 h at 37°C and cells were harvested by centrifugation.

After induction, cell cultures were centrifuged (8000 ×g for 20min) and the pellets were re-suspended in homogenization buffer (20mM Tris–Cl, 500mM NaCl, 10% glycerol, 50mM sucrose, pH 8.0). The cells were disrupted with a French Press (Constant Systems, Daventry, UK) at 27 Kpsi and the cell pellets were clarified by centrifugation (80,000 xg for 40 min). The induced rA21618-D47 was washed with 2M urea/PBS pH 7.2 and extracted from the cell pellet using 4M urea/PBS, pH 7.2. The expressed rA21618-SDSK was washed with homogenization buffer containing 3 M guanidine hydrochloride and extracted from the cell pellet using homogenization buffer containing 6 M guanidine hydrochloride. The extracted proteins were both purified using an IMAC column (Ni-NTA resin). On-column folding procedure was used to prepare the soluble form of rA21618-D47 and rA21618-SDSK free of chaotropic agents. LPS was removed by on-column wash with 0.1% of Triton X-114. The amount of residual LPS in rA21618-D47 and rA21618-SDSK were below 100 EU/mg. The endotoxin-free proteins were dialyzed against PBS for animal experiments.

To express rlipo-A21618-D47 and rlipo-A21618-SDSK, the PCR products of A21618-D47 and rA21618-SDSK were cloned into an expression vector, pET-22b (+) containing the D1 sequence from rAg473, using BamHI and XhoI sites. The expression plasmids were transformed into the *E. coli* C43 (DE3) strain. The transformed cells were grown aerobically overnight in LB medium at 37°C. The overnight culture was transferred to the M9 medium at a ratio of 1:25 and grown aerobically in the shaking incubator (200 rpm) at 37°C. The expression of rlipo-A21618-D47 and rlipo-A21618-SDSK was induced with 1mM IPTG at 20°C for 20 h and 25°C for 16 h respectively. The cells were harvested by centrifugation. The collected cells were re-suspended in homogenization buffer and disrupted by French Press (Constant Systems, Daventry, UK) at 27 kpsi. The cell pellets containing the induced rlipo-A21618-D47 and rlipo-A21618-SDSK were clarified by centrifugation (80,000g for 30 min) and extracted from the cell pellet using 20mM

Tris (pH 8.9)/1% Triton X-100 and 50 mM Tris (pH 8.9)/1% Triton X-100 respectively. The extracted proteins were purified using an IMAC column (Ni-NTA resin). Triton X-100 was washed out during the purification. LPS was removed by on-column wash with 0.1% of Triton X-114. The amount of residual LPS in rlipo-A21618-D47 and rlipo-A21618-SDSK were below 100 EU/mg. The endotoxin-free lipoproteins were dialyzed against PBS for animal experiments.

The amino acid sequences of rA21618-D47, rlipo-A21618-D47, rA21618-SDSK, or rlipo-A21618-SDSK are shown in Figs. 1 and 2.

Four groups of HLA-A2-transgenic mice (3 per group) were respectively administered subcutaneously with rA21618-D47, rlipo-A21618-D47, rA21618-SDSK, or rlipo-A21618-SDSK. Each mouse received two doses of 30 μg of each polypeptide in PBS buffer on Days 0 and 14. Mice were sacrificed on Day 21 and their spleens were collected. The spleens were meshed to generate a single cell suspension in RPMI 1640 medium. The cell suspension was centrifuged at 1500 rpm for 5 minutes to pellet the cells. The pellet was re-suspended in 10 ml of RPMI 1640 medium and red cells were lysed using erythrocyte lysing buffer (0.15 M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA) for 1 min at room temperature. Cells were then washed with RPMI 1640 medium and re-suspended in complete RPMI 1640 medium (RPMI 1640 medium supplemented with 10% FBS and 1% mixture of penicillin, streptomycin, and L-glutamate). Cell concentration in suspension was calculated and adjusted to a concentration of 5×10^6 cells per ml.

Splenocyte were plated at 5×10^5 cells per well and cultured with 10 μg/ml of peptides for 48 h, followed by a gamma interferon (IFN-γ) enzyme-linked immunospot (ELISPOT) assay (eBioscience, San Diego, CA). The wells of 96-well plates with nitrocellulose membrane inserts were coated with 50 μl of anti-IFN-γ antibody solution (clone AN18, 10 μg/ml in 1x PBS; eBioscience) and incubated for 18 h at 4°C. The plates were then washed with PBS twice and blocked with 100 μl of complete RPMI medium per well for 1 to 3 h to prevent nonspecific binding in later steps. Next, 5×10^5 splenocytes with 10 μg/ml of the peptides shown in Table 1 above were added to the plate at a final volume of 200 μl. The ELISPOT assay was performed in triplicate for each experimental condition. Specifically, the plates were incubated in a humidified atmosphere of 5% CO2 in air at 37°C for 48 h. After this incubation, the cells were

removed from the plate by washing three times with 0.05% (wt/vol) Tween 20 in PBS. A 50-μl aliquot of biotinylated anti-IFN-γ antibody (clone R46A2, 2 μg/ml in PBS; eBioscience) was then added to each well. The plates were again incubated at room temperature for 2 h. The washing steps were repeated. After 45 minutes of incubation with an avidin-HRP complex reagent (eBioscience) at room temperature, the plates were washed again three times with 0.05% (wt/vol) Tween 20 in PBS and then twice with PBS alone. A 100-μl aliquot of 3-amine-9-ethyl carbazole (AEC; Sigma-Aldrich, St. Louis, MO) staining solution was added to each well to develop the spots. The reaction was stopped after 4 to 6 min by placing the plate under tap water. The spots were then counted using an ELISPOT reader (Cellular Technology Ltd., Shaker Heights, OH). Both rlipo-A21618-SDSk and rlipo-A21618-D47 induced epitope-specific CTL response in HLA-A2-transgenic mice. See Figs. 3 and 4.

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

WHAT IS CLAIMED IS:

1. An isolated lipidated polypeptide comprising a lipid moiety at the N-terminus and a plurality of epitopes.

2. The polypeptide of claim 1, wherein each epitope of the plurality is different.

3. The polypeptide of claim 1, wherein all epitopes of the plurality are cytotoxic T lymphocyte (CTL) epitopes.

4. The polypeptide of claim 1, wherein all epitopes of the plurality are from a single pathogen.

5. The polypeptide of claim 4, wherein the pathogen is human papillomavirus (HPV).

6. The polypeptide of claim 5, wherein the plurality of epitopes include one or more epitopes on E6 or E7.

7. The polypeptide of claim 5, wherein the plurality of epitopes include different HLA-A2-restricted epitopes of HPV type-16 and type-18.

8. The polypeptide of claim 1, wherein the plurality of epitopes include one or more of the peptides listed in Table 1.

9. The polypeptide of claim 8, wherein the plurality of epitopes include all of the peptides listed in Table 1.

10. The polypeptide of claim 1, wherein the epitopes of the plurality are noncontiguous.

11. The polypeptide of claim 1, wherein the polypeptide is rlipo-A21618-D47 or rlipo-A21618-SDSk.

12. A composition comprising the lipidated polypeptide of any one of claims 1-11.

13. A method of producing the lipidated polypeptide of claim 1, the method comprising:

providing an *E. coli* host cell that has an expression vector including a nucleic acid sequence encoding a polypeptide, the polypeptide containing a bacterial lipoprotein signal peptide and the plurality of epitopes; and

culturing the *E. coli* host cell under conditions that allow expression of the polypeptide in lipidated form, thereby producing the lipidated polypeptide.

14. The method of claim 13, wherein the nucleic acid sequence is codon-optimized for expression in *E. coli*.

15. The method of claim 13, wherein the lipoprotein signal peptide includes the D1 domain of Ag473.

16. The method of claim 13, further comprising isolating the lipidated polypeptide.

17. Use of the composition of claim 12 for eliciting an immune response in a subject.

18. The use of claim 17, wherein the immune response is a CTL-mediated immune response.

19. Use of a composition containing the lipidated polypeptide of any one of claims 5-11 for treating an HPV-associated disease.

FIG. 1

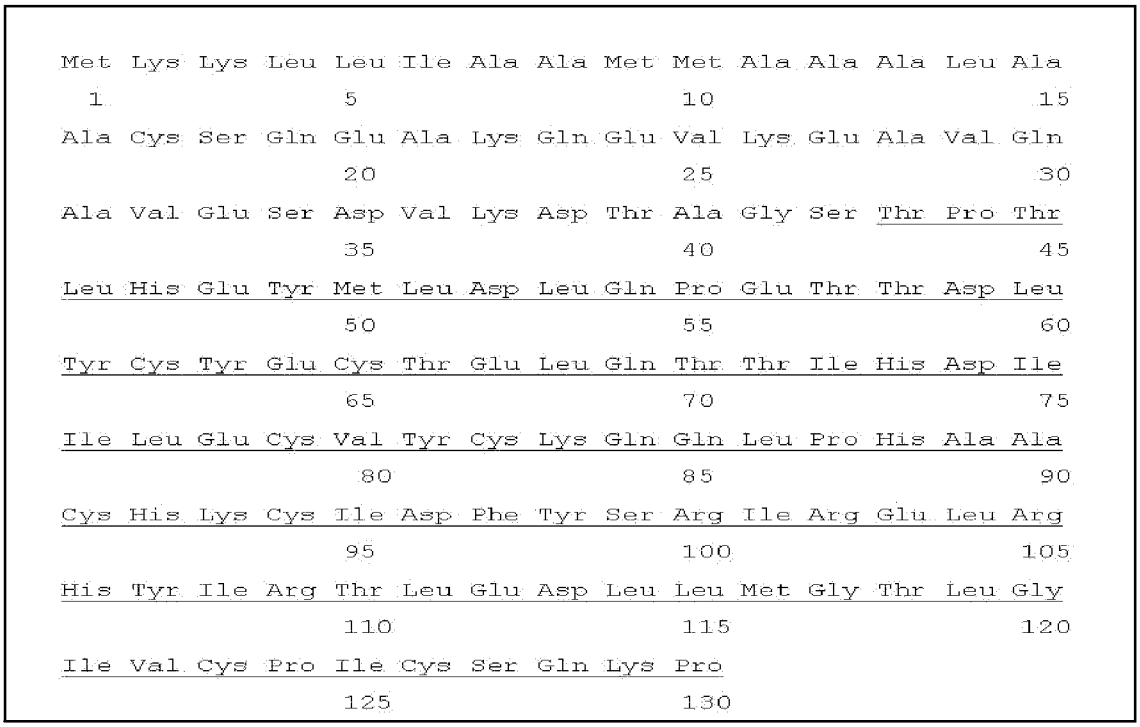

Met Lys Lys Leu Leu Ile Ala Ala Met Met Ala Ala Ala Leu Ala
1 5 10 15
Ala Cys Ser Gln Glu Ala Lys Gln Glu Val Lys Glu Ala Val Gln
20 25 30
Ala Val Glu Ser Asp Val Lys Asp Thr Ala Gly Ser Thr Pro Thr
35 40 45
Leu His Glu Tyr Met Leu Asp Leu Gln Pro Glu Thr Thr Asp Leu
50 55 60
Tyr Cys Tyr Glu Cys Thr Glu Leu Gln Thr Thr Ile His Asp Ile
65 70 75
Ile Leu Glu Cys Val Tyr Cys Lys Gln Gln Leu Pro His Ala Ala
80 85 90
Cys His Lys Cys Ile Asp Phe Tyr Ser Arg Ile Arg Glu Leu Arg
95 100 105
His Tyr Ile Arg Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly
110 115 120
Ile Val Cys Pro Ile Cys Ser Gln Lys Pro
125 130

FIG. 2

```
Met Lys Lys Leu Leu Ile Ala Ala Met Met Ala Ala Ala Leu Ala
 1               5                  10                  15
Ala Cys Ser Gln Glu Ala Lys Gln Glu Val Lys Glu Ala Val Gln
            20                  25                  30
Ala Val Glu Ser Asp Val Lys Asp Thr Ala Gly Ser Met Ser Ala
        35                  40                  45
Ala Lys Phe Val Ala Ala Trp Thr Leu Lys Ala Ala Ala Ser Asp
    50                  55                  60
Ser Gly Ile Leu Gly Phe Val Phe Thr Leu Lys Ala Ala Ser Ile
65                  70                  75
Ile Asn Phe Glu Lys Leu Ala Ala Ala Cys Thr Glu Leu Gln Thr
                80                  85                  90
Thr Ile His Asp Ile Ile Leu Glu Cys Val Tyr Cys Lys Gln Gln
            95                  100                 105
Leu Pro His Ala Ala Cys His Lys Cys Ile Asp Phe Tyr Ser Arg
        110                 115                 120
Ile Arg Glu Leu Arg His Tyr Thr Pro Thr Leu His Glu Tyr Met
    125                 130                 135
Leu Asp Leu Gln Pro Glu Thr Thr Asp Leu Tyr Cys Tyr Glu Ile
140                 145                 150
Arg Thr Leu Glu Asp Leu Leu Met Gly Thr Leu Gly Ile Val Cys
155                 160                 165
Pro Ile Cys Ser Gln Glu Asp Leu Leu Met Gly Thr Leu Gly Ile
            170                 185                 190
Val Cys Pro Ile Cys Ser Gln Lys Pro Ala Asp Asp Leu Arg Ala
        195                 200                 205
Phe Gln Gln Leu Phe Leu Asn Thr Leu Ser Phe Val Cys Pro Trp
    210                 215                 220
```

FIG. 3

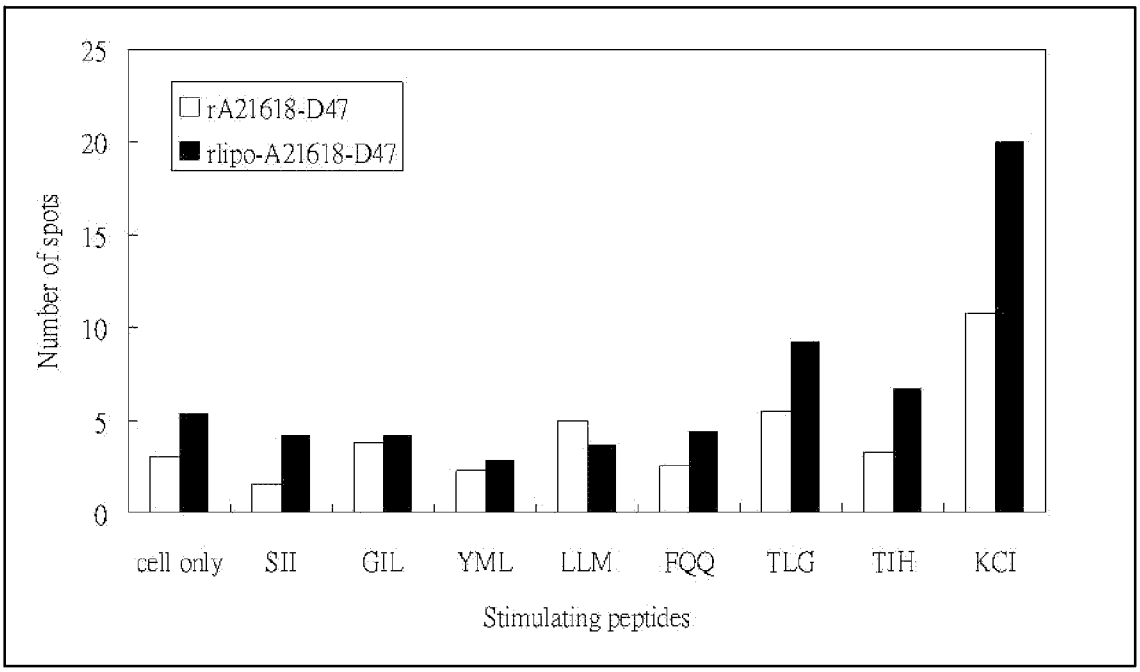
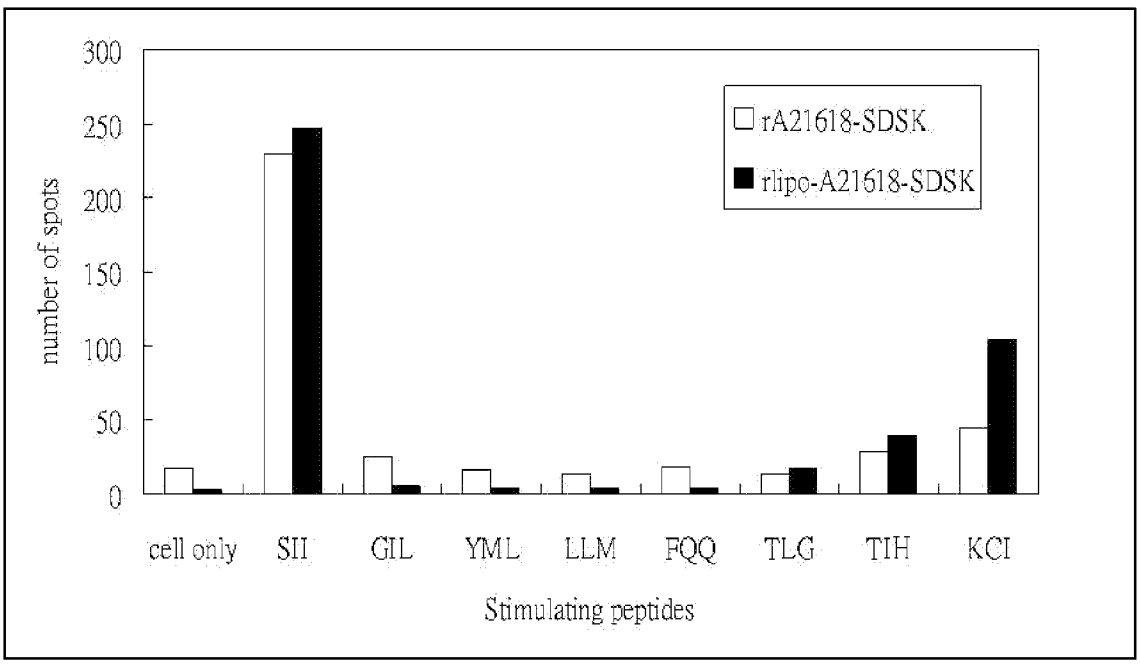

25
20
15
10
5
0
Number of spots
rA21618-D47
rlipo-A21618-D47
cell only
SII
GIL
YML
LLM
FQQ
TLG
TIH
KCI
Stimulating peptides

FIG. 4


300
250
200
150
100
50
0
number of spots
rA21618-SDSK
rlipo-A21618-SDSK
cell only
SII
GIL
YML
LLM
FQQ
TLG
TIH
KCI
Stimulating peptides

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002912

A. CLASSIFICATION OF SUBJECT MATTER

IPC: ***C07K 17/02*** (2006.01), ***A61K 39/12*** (2006.01), ***A61P 31/20*** (2006.01), ***C12N 15/00*** (2006.01), ***C12P 21/02*** (2006.01), *C07K 14/01* (2006.01), *C12N 15/70* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K 17/02 (2006.01), ***A61K 39/12*** (2006.01), ***A61P 31/20*** (2006.01), ***C12N 15/00*** (2006.01), ***C12P 21/02*** (2006.01), *C07K 14/01* (2006.01), *C12N 15/70* (2006.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

TotalPatent, PubMed, Canadian Patent Database, GenomeQuest. lipidated peptide, protein, vaccine, epitope(s), HPV, human papilloma virus, HPV 16/18, bacterial lipoprotein, Ag473, D47, SDSk, A21618

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, P	WO 2010/148496 A1 (HSIANG, C.-H. et al) 29 December 2010 (29-12-2010)	1-6, 8, 10, 12-19
X --- Y	US 2009/0081253 A1 (HANON, E.J. et al) 26 March 2009 (26-09-2009), Paragraphs [0110]-[0112], [0115], [0116], [0120], [0166]-[0177].	1-8, 10, 12-14, 16-19 9
X --- Y	WO 2004/052395 A1 (BIEMANS, R. et al) 24 June 2004 (24-06-2004), page 5, lines 3-15; page 8, line 26 to page 10, line 7.	1-8, 10, 12-14, 16-19 9
X --- Y	CHEN, W. et al, Induction of cytotoxic T-lymphocytes and antitumor activity by a liposomal lipopeptide vaccine. Mol. Pharm., 2008, Vol.5, No.3, pages 464-471, ISSN: 1543-8384, the whole document.	1-6, 8, 10, 12-14, 16-19 9

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 March 2012 (01-03-2012)	18 April 2012 (18-04-2012)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Riad Qanbar (819) 934-7937

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002912

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEN, H.W. et al. A novel technology for the production of a heterologous lipoprotein immunogen in high yield has implications for the field of vaccine design. Vaccine, 2009, Vol.27, No.9, pages 1400-1409, ISSN: 0264-410X, the whole document.	1-4, 10, 12-18
X	JACKSON, D.C. et al) A totally synthetic vaccine of generic structure that targets Toll-like receptor 2 on dendritic cells and promotes antibody or cytotoxic T cell responses. Proc. Natl. Acad. Sci. USA, 2004, Vol.101, No.43, pages 15440-15445, ISSN: 0027-8424, the whole document.	1-3, 8, 10, 12, 17, 18
Y	STELLER, M.A. et al, Cell-mediated Immunological Responses in Cervical and Vaginal Cancer Patients Immunized with a Lipidated Epitope of Human Papillomavirus Type 16 E7. Clin. Cancer Res., 1998, Vol.4, No.9, pages 2103-2109, ISSN: 1078-0432, the whole document.	9
Y	WO 92/16636 A1 (BOURSNELL, M.E.G. et al) 1 October 1992 (01-10-1992) the whole document, particularly, Figures 1a, 1b and 26.	9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002912

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons :

1. [ ] Claim Nos. :
because they relate to subject matter not required to be searched by this Authority, namely :

2. [ ] Claim Nos. :
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically :

3. [ ] Claim Nos. :
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows :

The claims are directed to a plurality of technical concepts as follows:

Group 1 - Claims 1-4, 8-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope SIINFEKL.

(continued on supplemental sheet)

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos. :

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos. :

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2011/002912

Continuation of Box (Unity of invention is lacking)

Group 2 - Claims 1-4, 8-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope GILGFVFTL.

Group 3 - Claims 1-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope YMLDLQPETT.

Group 4 - Claims 1-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope LLMGTLGIV.

Group 5 - Claims 1-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope FQQLFLNTL.

Group 6 - Claims 1-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope TLGIVCPI.

Group 7 - Claims 1-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope TIHDIILECV.

Group 8 - Claims 1-19 (all partially) feature a N-terminally lipidated peptide containing a plurality of epitopes including the epitope KCIDFYSRI.

The subject matter of groups 1 to 8 relates to a lipidated polypeptide comprising a lipid moiety at the N-terminus and a plurality of epitopes. However, such polypeptides were known in the art on the relevant date (see, for example, the X documents cited on pages 4 and 5 of this search report). There is no other technical feature common to all claims. Accordingly, each of groups 1 to 8 is considered a separate alleged invention.

The claims must be limited to one inventive concept as set out in Rule 13 of the PCT.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/IB2011/002912

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
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(Continued on next page)

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